PATENT APPLICATION

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Futoshi OKADA et al

For: ANTI-TUMOR AGENT

Serial No.: 10/655 567 Group: 1653

Confirmation No.: 6434

Filed: September 4, 2003 Examiner: Kosson

Atty. Docket No.: Furuya 1407

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

## DECLARATION UNDER 37 CFR 1.132

I, the undersigned, hereby declare as follows:

I am one of the co-inventors of the invention described and claimed in application Serial No. 10/655 567, filed on September 4, 2003.

I hereby incorporate by reference herein the contents of the Example contained on pages 6-10 of the above-identified application and the Declaration Under 37 CFR 1.132 dated April 13, 2005.

I have performed another test to illustrate the efficacy of SOD-G in inhibiting the malignant progression of human colonic adenoma cells.

An epithelial cell line designated a FPCK-1-1 was established from a colonic polyp in a male patient with familial adenomatous polyposis. The FPCK-1-1 cell line is non-tumorigenic when subcutaneously injected into mice in a cell suspension of up to $5 \times 10^6$ cells per nude mouse. Conversely, implantation of $1 \times 10^5$ of FRCK-1-1 cells attached to a plastic plate into mice first induced acute and then chronic inflammation, and formed progressively growing tumors that were histopathologically determined as moderately differentiated adenocarcinoma. The results are shown below.

Table 5 Inhibition of inflammation-promoted acquisition of tumorigenic ability of human colonic adenoma FPCK-1-1 cells in KSN nude mice by SOD-G

| Treatment a) | Tumorgenicity No. of mice with tumor/no. of mice injected b) | Mean latency period (days) | Histopathologic findings (Incidence) |
|---|---|---|---|
| None | 14/20 | 110 ± 5 | Moderately differentiated adenocarcinoma (14/14) |
| SOD | 15/20 | 103 ± 11 | Moderately differentiated adenocarcinoma (15/15) |
| SOD-G | 3/20 c) | 108 ± 10 | Moderately differentiated adenocarcinoma (3/3) |

a): Female KSN nude mice of 5-weeks old were fed with feed containing melon SOD or SOD-G at a concentration of 0.01% from 2 days before implantation of adenoma and until 120 days after the implantation.

b): FPCK-1-1 adenoma, $1 \times 10^6$ cells were attached to plastic palates of 10 × 5 × 1 mm pieces in vitro. The plates were implanted sc in the nude mice. One hundred and 20 days after implantation the plastic plate was removed and tumor tissue developed was fixed and stained with hematoxylin and eosin for histopathologic examination.

c): Statistic analysis by Fisher's test: SOD-G vs SOD or None, $p < 0.01$.

## DISCUSSION OF RESULTS

As shown by the results contained in Table 5, the oral administration of SOD-G to the mice effectively inhibited the malignant progression of human colonic adenoma in the mice as out of twenty mice implanted with $1 \times 10^5$ of FPCK-1-1 cells attached to a plastic plate, only three developed tumors. On the other hand, of the twenty mice orally administered melon SOD and implanted with $1 \times 10^5$ of FPCK-1-1 cells attached to a plastic plate, fifteen developed tumors.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: Jan. 11, 2006

Hiroshi Shionoya

Hiroshi SHIONOYA